The Omega-6/Omega-3 Ratio and Cardiovascular Disease Risk: Uses and Abuses

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The cardioprotective effects of omega-3 fatty acids (n-3 FAs) are well known, but the role that the n-6 FAs play in coronary heart disease is unclear. These two classes of essential FAs compete for a number of enzyme systems, and their metabolites can powerfully influence (often in different directions) inflammatory responses, vascular reactivity, and platelet aggregation. Accordingly, the n-6/n-3 FA ratio may be of value in interpreting biomarker data and in making nutritional recommendations. Although initially appealing, there are few human experimental and clinical trial data to support this view. This paper reviews a variety of studies that, in the aggregate, suggest that the ratio is, both on theoretical and evidential grounds, of little value. Metrics that include the n-3 FAs alone, especially eicosapentaenoic and docosahexaenoic acids, appear to hold the greatest promise.

Introduction

I recently moved to Sioux Falls, SD. On one sunny Saturday morning, my wife visited a farmers' market where local "organic" produce and meats were being offered. She picked up a copy of the *Goosemobile News*, a newsletter extolling the benefits of meat from free-range animals. The author wrote, "Omega-3 fatty acids (good) and omega-6 fatty acids (bad) are more balanced in grass-fed beef." This "grass-roots" comment reflects the perception that our diets suffer from an "omega imbalance," an unhealthy omega-6/omega-3 (n-6/n-3) ratio that can be remedied by eating more of the "good" and less of the "bad" fats. This view is shared by some nutrition scientists [1–4]. Simopoulous [5] states that "The ratio of omega-6 to omega-3 fatty acids is an important determinant of health," and de Lorgeril and Salen [6] have written that "From more recent studies we are learning that the omega-6/omega-3 ratio is important for the prevention of CHD."

The purpose of this paper is to discuss the potential value of the n-6/n-3 fatty acid (FA) ratio (as either biomarkers or in the diet) as a target for reducing risk for coronary heart disease (CHD). The fundamental question with respect to CHD is this: is risk for CHD reduced more effectively by targeting a specific dietary n-6/n-3 FA ratio or by targeting specific dietary intakes for n-6 and n-3 FAs? I, like others [7,8], argue for the latter.

In addressing this question, we must consider corollary assumptions that are implicit in "ratio" thinking: 1) that current dietary intakes of n-6 FA are excessive and detrimental to cardiac health; 2) that eating less n-6 FA (ie, linoleic acid [LA]) will reduce levels of the primary offending n-6 FA (arachidonic acid [AA]) in the tissues; and 3) that higher intakes of n-3 FA will cancel the adverse effects of n-6 FA.

Metabolic Inter-relationships

Recommendations to lower the n-6/n-3 ratio are based, in part, on the well-known competition between linoleic acid (C18:2n6) and α-linolenic acid (ALA, C18:3n3) for the Δ6-desaturase, which converts them to γ-linolenic acid (C18:3n6) and stearidonic (C18:4n3) acids, respectively (Fig. 1). These FAs are then converted into the n-6 FA arachidonic acid (C20:4n6) and the n-3 FA eicosapentaenoic acid (EPA, C20:5n3) by the Δ5-desaturase, and on to docosapentaenoic acid (C22:5n6) and docosahexaenoic acid (DHA, C22:6n3), respectively, by a series of elongations, Δ6-desaturation, and β-oxidation. Once in tissue membranes, AA and EPA compete for release by phospholipases and for conversion into a myriad of potent regulatory eicosanoids. These molecules include prostanoids (prostaglandins, thromboxanes, and prostacyclins derived from cyclooxygenase), leukotrienes and lipoxins (derived from lipoxygenase), endogenous cannabinoids

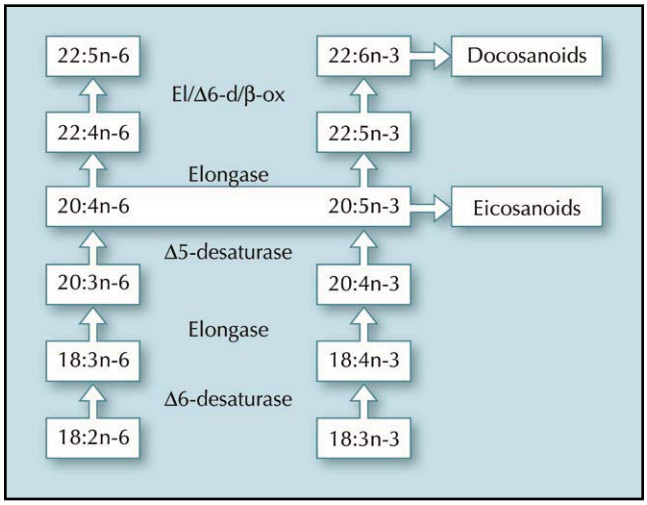

Figure 1. Essential fatty acid metabolism. Linoleic (C18:2n-6) and α-linolenic (C18:3n-3) acids compete for the Δ6-desaturase as their first step towards the production of their bioactive 20-carbon metabolites, arachidonic acid (C20:4n-6) and eicosapentaenoic (C20:5n-3) acid. Further competition occurs at the Δ5-desaturase step.

(anandamide, 2-arachidonyl-glycerol), resolvins and neuroprotectins (derived from aspirin-blocked cyclooxygenase-2), docosanoids (derived from DHA), and epoxyeicosatrienoic acids. The 20- and 22-carbon essential FAs also affect metabolism indirectly by altering the composition (and thus the function) of cellular membranes (lipid rafts), which can impact the activities of membrane-bound proteins. Finally, they can influence gene expression by activating or inhibiting the synthesis of a variety of transcription factors. Thus, the n-6 and n-3 eicosanoids have widespread influence in cellular metabolism.

Some of the eicosanoids derived from AA are more potent than those derived from EPA (eg, prostaglandin E_2 vs E_3; thromboxane A_2 vs A_3; leukotriene B_4 vs B_5). Others share a similar potency (eg, prostacyclin [PGI_2] and PGI_3). In general, however, replacement of AA with EPA (which would reduce the membrane AA/EPA ratio), dampens proinflammatory and proaggregatory responses, at least in vitro and in animal models [9,10]. The AA/EPA ratio is a potentially more relevant form of the n-6/n-3 ratio because it focuses specifically on the two molecules that compete for conversion to bioactive eicosanoids. But, as we see in the following text, it still suffers from the mathematical weaknesses of ratios.

Circumstantial Evidence for Adverse Cardiovascular Effects of Higher n-6 FA Intakes

Diets high in n-6 FAs increase the susceptibility of low-density lipoproteins (LDL) to oxidative modification [11], an effect that could be considered proatherogenic. Unfortunately, the n-3 FAs can have the same or greater effect [12], and yet are clearly antiatherogenic. This somewhat diminishes the force of the oxidation argument. Also brought to bear in service of this hypothesis is the observation that diets rich in n-6 FAs can diminish the rise in erythrocyte membrane EPA content following fish oil supplementation [13].

Higher intakes of AA and n-6 FAs have recently been associated with an increase in carotid intimal-medial thickness (IMT) in individuals with a variant allele in the promoter region of the gene for 5-lipoxygenase (5-LOX), the rate-controlling enzyme for leukotriene synthesis [14]. Here, the link between higher intake of n-6 FA and a surrogate marker of CHD risk appears clear. It must, however, be appreciated that the relationship between AA and IMT was only observed in that 6% of the study population with the 5-LOX variant; there was no relationship with n-6 FA intake whatsoever in the vast majority with normal 5-LOX function. (Interestingly, the relationship between AA intake and IMT in the variant subgroup was not seen when intakes of long-chain n-3 FAs were higher).

Finally, the transfection into both mice [15••] and pigs [16] of the *fat-1* gene from *Caenorhabditis elegans* (which encodes for the n-3 desaturase that enables the direct conversion of n-6 FAs to n-3 FAs in vivo) creates a marked decrease in tissue n-6/n-3 ratios. Although undoubtedly an impressive technologic achievement, the relevance of this finding to human disease remains to be determined. There is no a priori reason to conclude that organisms with this genetic implant are somehow "seeking" a tissue FA composition that, in some teleologic sense, affords the organism "optimal" health. As a means of producing genetically modified foods enriched with n-3 FAs, this technology appears to hold great promise, but how the observations per se inform the question of what human tissue FA composition should or could be is unclear. These and other arguments are marshaled to advocate use of the n-6/n-3 FA ratio both in making dietary recommendations and in interpreting biomarker data. The following discussion highlights conceptual concerns with the ratio as well as experimental and epidemiologic findings relevant to this question.

Theoretical Concerns with Ratios

There are multiple ways in which an elevated (ie, "bad") n-6/n-3 FA ratio could theoretically be produced, but the clinical significance of each would likely be very different. For example, depending on how the proportion of n-6 FAs changed, the proportions n-3 FAs could decrease, not change, or actually increase, and in each case result in a higher ratio (Table 1). Based on this consideration alone, such a metric should be highly suspect. Without knowledge of the absolute values of the numerator and the denominator, the meaning of a given ratio, whether as a biomarker or dietary target, will be impossible to discern. It is not uncommon for investigators to report the relationship between an intervention or an outcome of interest and the n-6/n-3 ratio without first considering (or even reporting) whether relationships existed with the n-6 FAs or the n-3 FAs alone [17,18]. To be of incremental value, a ratio must provide greater discriminatory

power than either component alone. If all of the discriminatory power of the ratio derives from the denominator alone (as appears to be the case for the n-6/n-3 ratio), then blending the weaker predictor with the stronger serves only to diminish the overall predictive potential of the latter. In addition, as any ratio is comprised of two measurements, each with its own analytical error, it is in principle likely to be a less precise metric than either component alone.

A second important conceptual limitation of the n-6/n-3 FA ratio is its failure to distinguish between the long- and short-chain FA within each class (ie, between the n-3 FAs, ALA, and EPA/DHA, and the n-6 FAs, LA, and AA) (Table 2). In both cases, the 18-carbon species have clearly different physiologic properties than the 20- or 22-carbon species on both platelet function [19] and triglyceride lowering [20,21]. Accordingly, the failure of the n-6/n-3 FA ratio to distinguish specific FAs is a fundamental conceptual flaw.

Finally, ratios are of value only if two conditions are met. First, higher levels of one factor and lower levels of the other should each be predictive of increased risk. For example, consider the LDL/high-density lipoprotein (HDL) ratio. Here, higher levels of LDL and lower levels of HDL increase CHD risk, thus combining them into a ratio has some merit. However, as discussed in the next section, higher intakes and tissue levels of n-3 and n-6 (to a lesser extent) FA are both associated with decreased CHD risk; therefore, the logic of bundling them into a ratio is flawed.

However, despite all of these conceptual problems, if direct evidence could be found that an elevated ratio (independent of the individual n-6 and n-3 FA values) imparted increased risk for CHD, then these theoretical concerns could be dismissed. In the sections that follow, clinical trial and human experimental data are considered that bear on this question.

Table 1. Five ways to lower an elevated omega-6/omega-3 fatty acid ratio

Omega-6 fatty acid	↓	→	↑	↓	↓↓
Omega-3 fatty acid	↑	↑	↑↑	→	↓

Table 2. Theoretical fatty acid intakes that produce the same omega-6/omega-3 ratio*

n-6/n-3 ratio	5	5	5	5	5	5	5
LA							
% en	20	20	10	10	10	2	2
g/d	44	44	22	22	22	4.4	4.4
ALA							
% en	4	0	2	0	1	0.4	0
g/d	8.8	0	4.4	0	2.2	0.88	0
EPA and/or DHA							
% en	0	4	0	2	1	0	0.4
g/d	0	8.8	0	4.4	2.2	0	0.88

*Values are based on a total energy intake of 2000 kcal/d.
% en—percent of total energy; ALA—α-linolenic acid; DHA—docosahexaenoic acid; EPA—eicosapentaenoic acid; LA—linoleic acid; n-3—omega-3; n-6—omega-6.

Dietary FA and CHD: Epidemiologic Evidence

The contention that high intakes of n-6 FAs, because they give rise to proinflammatory, proaggregatory, and vasoconstrictive eicosanoids, predispose to CHD events enjoys little clinical confirmation. Data from studies examining the relationship between n-6 and n-3 FA intakes and biomarkers on the prevalence and the incidence of CHD are presented in the following text.

In the National Heart, Lung and Blood Institute (NHLBI) Family Heart Study, Djousse et al. [22] examined the relationships between dietary LA and ALA on the prevalence of coronary artery disease (CAD) in over 4500 participants with a mean age of 52 years. They concluded that "A higher intake of either ALA or LA was inversely related to the prevalence odds ratio of CAD. The two FA had synergistic effects…"

The relationship between FA intakes and incidence of CHD events was studied by Mozaffarian et al. [23], who asked whether higher n-6 FA intakes blunted the beneficial effects of higher n-3 FA intakes on risk for CHD events in the Health Professionals Follow Up Study (HPFS). They first reported that men with long-chain n-3 FA intake above the median (> 250 mg/d) had a lower risk for sudden cardiac death than those men with an intake below the median (hazard ratio of 0.52; 95% CI, 0.34–0.79). Importantly, this reduction in risk was identical for individuals consuming LA above or below median levels (11.2 g/d). The same situation held true for ALA, with higher intakes being cardioprotective (hazard ratio of 0.89; 95% CI, 0.78–0.99) regardless of background n-6 FA intakes. These findings, which derive from data from over 51,000 men followed for 14 years, are not consistent with the view that the n-6/n-3 ratio has clinical relevance beyond the relevance of the denominator alone.

At least three prospective cohort studies found that higher LA intakes were protective, not detrimental, against CHD. In an earlier analysis from the HPFS [24], the risk for CHD was inversely correlated with the linoleic acid intake, with a 5% increase in intake being associated with a relative risk of 0.58 ($P < 0.05$). In the Nurses' Health Study, Hu et al. [25] reported that an increase in the intake of linoleic acid from the lowest quintile (2.9% energy) to the highest (6.4% energy) was associated with a 32% reduction in risk for CHD events (P for trend = 0.003). Finally, risk for death from CHD was 34% higher in that third of the population consuming the lowest amounts of polyunsaturated FA (PUFA) compared with the third consuming the highest ($P = 0.01$) in the Western Electric Study [26].

The strengths of these studies are their large sample sizes and long follow-up times, but their weaknesses are their observational nature and the possibility of confounding (ie, the reduced CHD rates derive from other unmeasured factors also associated with increased LA intake). Randomized prospective trials offer the possibility of determining cause-effect relationships, but the possibility of confounding remains, especially in studies where one component of the diet is, of necessity, substituted for another. In this case, saturated fat and cholesterol intakes were reduced to accommodate increased PUFA intakes.

Randomized Controlled Trials of Dietary FA and CHD

The hypothesis that higher LA is good, not bad, for the heart enjoys support from at least four randomized controlled trials (reviewed by Sacks and Katan [27]). In all four trials [28–31], the intake of LA and ALA was increased and total CVD events were reduced by 34%, 25%, 43%, and 12%, respectively. Only the latter was not statistically significant. These trials do not lend support to the view that higher intakes of n-6 FAs increase risk for CHD, and by extension, that higher n-6/n-3 ratios are harmful.

Serum FA and Inflammatory Markers

If the n-6/n-3 ratio logic holds, then one would predict that higher ratios (higher n-6 and/or lower n-3) in serum would be associated with increased levels of inflammatory markers. A number of groups have examined the relationships between either dietary FA intakes or serum FA content and markers of inflammation. For example, Lennie et al. [32] compared reported dietary intakes of FAs and serum levels of tumor necrosis factor-α (TNF-α) and two of its soluble receptors in 42 heart failure patients. Higher intakes of saturated FAs (above vs below 18 g/d) and trans FAs (above vs below 3.3 g/d) were associated with increased levels of TNF-α ($P < 0.05$). However, higher intakes of PUFA (over 90% n-6 FAs; above vs below 6% energy) and higher intakes of n-3 FAs (above vs below 1.2 g/d) were associated with lower levels of both of the soluble receptors ($P < 0.05$ for both).

Correlations between the habitual intake of essential FAs and serum inflammatory markers were also reported by Pischon et al. [33] in both the HPFS and the Nurses' Health Study. Although confirming that there was an inverse relationship between the intake of EPA and DHA and the two soluble receptors for TNF-α, they found no relationship between n-6 FAs and inflammatory markers. Interestingly, those individuals reporting the highest intakes of LA (> 6% energy) and of EPA and DHA (> 420 mg/d) had the lowest levels of both circulating receptors. These investigators concluded that "n6 FA do not inhibit the anti-inflammatory effects of n3 FA" and that "the combination of both types of FA is associated with the lowest level of inflammation."

A recent epidemiologic investigation conducted in two small towns in Tuscany also explored the relationships between plasma FAs and inflammatory biomarkers [34•]. Ferrucci et al. [34•] analyzed 1123 blood samples from both men and women across a spectrum of ages, but focused particularly on those over 65 years of age (78% of the sample). A multivariable statistical model relating inflammatory mediators to plasma FA composition revealed no relationships between any FAs and soluble interleukin-6 receptor (IL-6r) or TNF-α (Table 3). Decreased IL-6 and IL-1 receptor antagonist (ra), both considered proinflammatory markers, and increased transforming growth factor-β (TGF-β, an anti-inflammatory marker), were observed with increasing serum levels of AA, EPA, and DHA. Accordingly, the ratio of AA/EPA was not associated with any marker, either pro- or anti-inflammatory. The only 18-carbon essential FA to show a relationship with any inflammatory marker was ALA, which was negatively associated with the proinflammatory marker IL-1β.

When considered by n-6 or n-3 class, both classes of essential FAs were related to each inflammatory marker in generally the same way, and in no case were they at odds with each other (Table 3). For example, both FA classes were inversely associated with IL-6, IL-1ra, C-reactive protein, and TNF-α, and directly associated (either significantly so or as a trend) with the anti-inflammatory cytokines IL-6r, TGF-β, and IL-10. There were, however, four markers that were significantly associated with the n-6/n-3 ratio (Table 3). There are two reasons for this. For IL-6 and IL-10 the ratio was significantly related to these markers because the denominator, not the numerator, was. For IL-1ra and for TGF-β, the n-3 and the n-6 FAs were both associated in the same direction, but because the relationship was stronger for the n-3 than the n-6 FAs, the ratio of the two was altered. (The same phenomenon was observed in the case-control studies discussed in the following section.)

The findings of this study from Tuscany must be contextualized before being generalized. The dietary intake of n-6 PUFAs in this region of Italy is reported to be approximately 3.3% energy, a relatively low intake compared with other western countries, where mean intakes may be twice as high. Hence, these findings do not necessarily mean that at higher levels n-6 FAs are not proinflammatory. However, case-control studies conducted in a variety of places (North America, northern Europe, Israel, and Costa Rica) with higher LA intakes than those seen in Tuscany have also failed to demonstrate a link between higher n-6 FA levels and risk for CHD.

FA Biomarkers and Risk for CHD Events

Miettinen et al. [35] reported in 1982 that a higher serum LA content at baseline was inversely related to

Table 3. Multivariable analysis of the relationship between quartiles of individual n-6 and n-3 fatty acids, classes and ratios, and plasma levels of inflammatory markers

Marker	Pro- or anti-inflammatory	LA	AA	ALA	EPA	DHA	AA/EPA	n-3	n-6	n-6/n-3
IL-6	Pro	–	↓*	–	↓	↓	–	↓		↑*
Soluble IL-6r	Anti?	–	–	–	–	–	–	↑		
IL-1b	Pro	–	–	–	–	–	–			
IL-1ra	Pro	–	↓	↓	–	↓	–	↓	↓	↑
TNF-α	Pro	–	–	–	–	–	–	↓		
IL-10	Anti	–	–	–	–	↑	–	↑		↓
TGF-β	Anti	–	↑	–	↑	↑	–	↑	↑	↓
CRP	Pro	–	–	↓	–	–	–			

*Up and down arrows indicate a statistically significant ($P < 0.05$) positive and negative, respectively, relationship with the indicated marker across plasma fatty acid quartiles. For example, the down arrow under AA for IL-6 means that there was a significant negative (or inverse) correlation between serum levels of IL-6 and AA; higher AA was associated with lower IL-6. Variables included were age; sex; years of education; intake of total energy and of energy from carbohydrate, protein and fat; physical activity; smoking; low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations; body mass index; the presence or history of hypertension, diabetes, coronary heart disease, stroke, congestive heart failure, or peripheral artery disease; and potentially confounding drug treatment.
AA—arachidonic acid; ALA—α-linolenic acid; CRP—C-reactive protein; DHA—docosahexaenoic acid; EPA—eicosapentaenoic acid; IL—interleukin; LA—linoleic acid; n-3—omega-3; n-6—omega-6; ra—receptor antagonist; TGF-β—transforming growth factor-β; TNF-α—tumor necrosis factor-α.
(*Adapted from* Ferrucci et al. [34•].)

the 5-year risk for CHD. The findings from this and 12 other studies examining the ability of FA biomarkers to discriminate between patients at increased risk for CHD events and control subjects were recently published [36•]. Overall, the largest and most consistent difference between patients and control subjects was for the sum of EPA plus DHA, which was on average 10.8% lower in patients ($P = 0.002$) (Fig. 2). Total n-3 FAs were also lower ($P = 0.02$) in patients compared with control subjects. Interestingly, AA levels tended to be lower (not higher) in patients than in control subjects (-8.5%) as did the total n-6 FAs (-3.8%). Because the n-3 FA differential (-7.3%) was greater than that for the n-6 FA differential, the n-6/n-3 ratio was actually higher (5.6%; $P = 0.026$) in patients versus control subjects. This study highlighted three points: 1) AA levels are not higher in CHD patients versus healthy control subjects; 2) the discriminatory power of the n-6/n-3 FA ratio resided with the denominator; and 3) the inclusion of n-6 FAs in a ratio with the n-3 FAs served only to dilute and obscure the clearer signal provided by the latter (particularly EPA plus DHA).

Dietary Control of Tissue Essential FA Levels

If the n-6/n-3 FA ratio in tissues (particularly the ratio of the highly unsaturated FA [HUFA] of AA and EPA plus DHA) were of greater clinical significance than the n-3 FA level alone, and if decreasing dietary LA would reduce tissue AA levels, then the latter might be a reasonable approach to reducing risk for CHD. This has been proposed in Japan [37]. Besides the fact that there is no evidence that reducing n-6 FA intakes will reduce risk for CHD, controlled feeding studies in humans do not indicate that reducing LA intakes (except to levels of frank deficiency, less than about 0.6% energy [38]) will reduce tissue AA content. For example, Raatz et al. [39] fed LA at either 6% or 12% energy for 4 weeks to 10 volunteers and reported no difference in plasma phospholipid n-6 HUFAs. In fact, AA proportions tended to be higher on the lower LA diet. In a 6-month study conducted in four groups of volunteers given LA at 1.75%, 2.3%, 3.4%, or 6% of energy, neither erythrocyte nor platelet AA proportions were related to LA intake [40]. These findings suggest that across a wide range of LA intakes, tissue n-6 HUFAs are not materially altered. The American Heart Association [41] and the 2005 US Dietary Guidelines [42] continue to recommend LA intakes in the range of 5% to 10% of energy based on evidence that higher LA intakes can reduce, not increase, risk for CHD. Thus, dietary advice to lower n-6 FA intakes in order to reduce risk for eicosanoid-mediated, atherothrombotic, and other inflammatory diseases lacks a strong evidential basis.

Assuming that reducing the tissue content of AA is a laudable goal, and that this cannot be achieved in practice by decreasing LA intakes, how then can it be realized? The answer is by increasing the intake of EPA plus DHA. Increasing the intake of the n-3 HUFAs by 1, 2, or 3 g/d (about 0.5%, 1%, and 1.4% of energy) in a typical western diet containing 4% to 5% LA decreased the erythrocyte n-6 HUFAs (expressed as a percent of total HUFAs) from 62% at baseline to 46%, 38%, and 32%, respectively [43]. Thus, consuming relatively small amounts of n-3

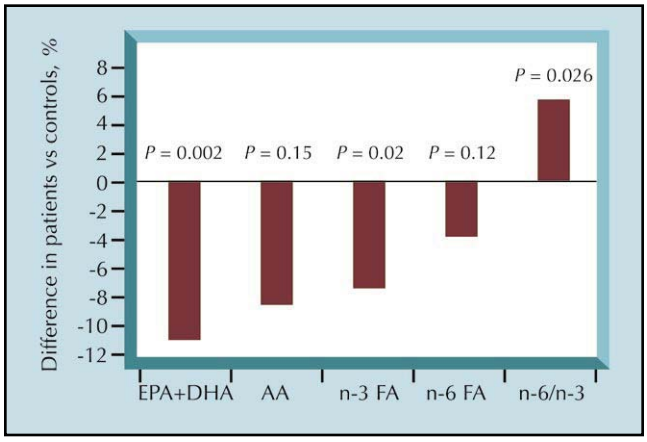

Figure 2. The percent difference in fatty acid biomarker values in coronary heart disease patients relative to healthy control subjects. A negative value means that the biomarker was lower in patients compared with control subjects. *P* values reflect the significance of each case-control comparison. AA— arachidonic acid; FA—fatty acid; DHA— docosahexaenoic acid; EPA— eicosapentaenoic acid; n-3—omega-3; n-6—omega-6. (*Adapted from* Harris et al. [36•].)

HUFAs is a far more efficient way to lower n-6 HUFA tissue proportions than decreasing LA intake from 13% to 2% energy. In addition, increasing n-3 FA intakes has been shown to reduce CHD events whereas reducing n-6 FA intakes has not.

Conclusions

Although an appealing concept at first glance, the n-6/n-3 ratio lacks many of the characteristics of a useful metric, both for interpreting biomarker data and for making dietary recommendations. It is conceptually flawed by obscuring absolute levels and chain-length differences within each FA class. There is virtually no evidence that lowering n-6 FA intakes (which will "improve" the ratio) will result in reduced risk for CHD, whereas increasing intakes of the n-3 FA will, regardless of the levels of n-6 FAs. Focusing on the ratio distracts from the more important issue, which is increasing the intake of the n-3 FAs. The latter appears to be the single most important dietary change that can be made to improve cardiovascular health.

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